CD44. If, as this study suggests, the interaction of CD44 with the extracellular environment does not play a role in effector T cell migration, it appears that during effector differentiation, T cells develop a dependency on CD44 to polarize. This conclusion could be tested by investigating the migration of effector T cells in lymph nodes and examining whether the tissue environment contributes to the differential behavior of naive and effector cells.

Beyond providing new insights into the biology of CD44, this study also makes an important contribution by highlighting the relevance of events that take place in peripheral tissues subsequent to recruitment from the blood in determining the outcome of T cell responses. It is conceivable that apart from the ability to migrate properly, T cells need to execute many other cellular functions that are subjected to extrinsic regulation in order to carry out their tasks.

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Elite Suppression of HIV-1 Replication

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Rare HIV-1-infected individuals are able to control viral replication without antiretroviral therapy. In this issue of *Immunity*, Migueles et al. (2008) show that HIV-1-specific $CD8^+$ T cells from these individuals effectively suppress viral replication by granzyme B-mediated killing of target cells.

HIV-1 normally replicates vigorously in untreated patients leading to global immune activation, progressive $CD4^+$ T cell depletion, and eventually frank AIDS. This progressive course is not seen in a select group of untreated patients who suppress viral replication to levels below the limit of detection of commercial assays. These individuals, who represent less than 0.5% of all HIV-1-infected patients, have been called elite controllers, elite suppressors, or HIV controllers (Deeks and Walker, 2007). In this issue of *Immunity*, Migueles et al. (2008) refer to them simply as long-term nonprogressors, but this term has traditionally been used to describe patients who maintain relatively high $CD4^+$ counts for prolonged periods of time regardless of the level of viral replication. The term HIV controllers (HCs) will therefore be used in this preview. Understanding the mechanisms responsible for the remarkable control of viral replication in these patients will obviously have major implications for the design of effective HIV-1 vaccines.

In a recent study, replication-competent HIV-1 was isolated from some HCs, and detailed genotypic and phenotypic analyses strongly suggested that these isolates were fully virulent (Blankson et al., 2007). Furthermore, a case of transmission of HIV-1 from a patient who developed AIDS to a subject who has been an HC for 10 years has recently been reported (Bailey et al., 2008). This provides strong evidence that unique host factors, and not infection with attenuated HIV-1 isolates, can explain the elite control of HIV-1 replication in at least some of these individuals. The search is on to determine exactly what these protective host factors are. The most consistent finding in studies of HCs is that certain class I HLA alleles such as HLA-B*57 and HLA-B*27 are overrepresented in HCs compared to both the general population and cohorts of HIV-1-infected patients with progressive disease (Deeks and Walker, 2007). Furthermore, whole-genome association scan analysis of HIV-1-infected individuals has shown that the factors most strongly associated with protection against disease are either HLA alleles (Fellay et al., 2007; Catano et al., 2008) or a single-nucleotide polymorphism that is in linkage disequilibrium with HLA-B*5701 (Fellay et al., 2007). Taken

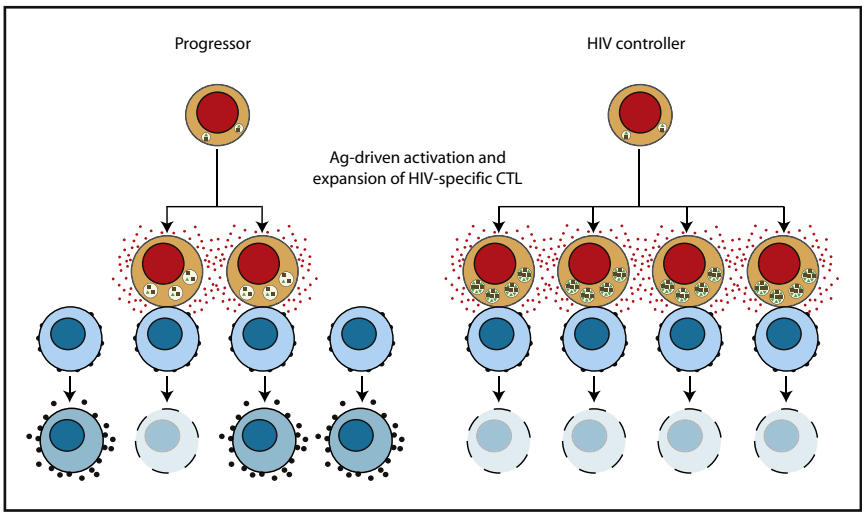

Figure 1. Response of HIV-1-Specific CD8$^+$ T Cells in Progressors versus HIV Controllers
After stimulation with HIV-1 antigen, CD8$^+$ T cells (orange) from both groups of patients secrete substantial amounts of IFN-γ (red dots), but efficient proliferation and clonal expansion is seen only in HIV controllers. In addition to proliferation, HIV-1-specific CD8$^+$ T cells from HIV controllers contain more granzyme B and perforin than do those from progressors (depicted as granules within CD8$^+$ T cells). This leads to dramatic killing of HIV-1-infected target CD4$^+$ T cells (blue) before the virus (black dots) can complete its life cycle. In progressors, lower amounts of granzyme B and perforin may be the cause of inefficient killing of HIV-1-infected CD4$^+$ T cells.

together, these results suggest that certain class I HLA molecules play a major role in HC status. It should be noted, however, that some HCs have none of the protective alleles, and some patients who do have these alleles develop progressive HIV-1 disease. Thus, although these host alleles are clearly important, they are neither necessary nor sufficient for elite suppression of viral replication.

One way that class I HLA molecules may directly contribute to the outcome of HIV-1 infection is by the presentation of processed peptide antigens to CD8$^+$ T cells. It has been known for many years that these effector cells are important for the initial partial control of HIV-1 replication after the peak viremia in primary infection. However, there is no correlation between the frequency of HIV-1-specific CD8$^+$ T cells and the degree of viremia present in the chronic phase of infection. Although studies of HIV-1-specific CD8$^+$ T cells have relied mostly upon assays that measured IFN-γ secretion in response to stimulation with processed peptides, recently developed assays have shown important qualitative differences in HIV-1-specific CD8$^+$ T cell function in HCs versus chronic progressors (CPs). For example, there is a dramatic difference in the proliferative capacity of CD8$^+$ T cells specific for an immunodominant epitope in the HIV-1 gag protein between HLA-B*57-positive HCs and CPs (Migueles et al., 2002). This enhanced proliferation was associated with expression of the lytic granule protein perforin, which plays a central role in the mechanism by which CD8$^+$ T cells kill virally infected target cells. In addition, CD8$^+$ T cells from HCs are more likely to secrete multiple cytokines in response to stimulation with HIV-1 peptides whereas those from CPs generally secrete only IFN-γ or MIP1β (Betts et al., 2006). A more recent study show that unstimulated CD8$^+$ T cells from HCs are able to dramatically suppress HIV-1 replication in autologous CD4$^+$ T cells, whereas those from CPs could not (Saez-Cirion et al., 2007).

These studies all underscore the point that the differences between the CD8$^+$ T cell responses of HCs and CPs are qualitative rather than quantitative in nature, but it is not clear how these differences translate to the effective control of viral replication in HCs. Proliferation would be expected to increase the frequency of effector cells, but, as noted above, more HIV-1-specific CD8$^+$ T cells does not necessarily mean better control of viremia. Similarly, cytokines such as IFN-γ, TNF-α, and IL-2 have not been shown to inhibit HIV-1 replication directly. Other studies have shown that the ability of unstimulated CD8$^+$ T cells to inhibit viral replication is contact dependent and thus not due to the secretion of soluble factors (Saez-Cirion et al., 2007).

In this issue, Migueles et al. (2008) perform a series of elegant experiments that further our understanding of the mechanisms involved in the control of viral replication. The investigators looked at the cytotoxic potential of CD8$^+$ T cells that had been freshly isolated from HCs and CPs versus cells from these subjects that had been stimulated in vitro with HIV-1 antigens for 6 days. They found a marked increase in killing of autologous CD4$^+$ target cells that were pulsed with immunodominant HIV-1 peptides by stimulated CD8$^+$ T cells from HCs but not CPs (Figure 1). HIV-1-specific CD8$^+$ T cells from HCs proliferated to a greater degree than those of CPs in response to in vitro stimulation with HIV-1 antigen, resulting in a much higher frequency of effector cells in HCs. Although there was no difference in the degranulation capacity of stimulated HIV-1-specific CD8$^+$ T cells from HCs versus CPs, a greater percentage of cells from HCs contained perforin and granzyme B.

A recently developed flow cytometric assay that measures granzyme B-mediated substrate cleavage in target cells was used to further investigate the observed cytoxicity. This assay allowed the investigators to determine the degree of exocytosis of functional granzyme B as opposed to the amount of total granzyme B present in the effector cells. It is more quantitative than the traditional chromium release assays because it allows the independent visualization of individual target cells and effector cells (by tetramer staining). Stimulated HIV-1-specific CD8$^+$ T cells from HCs generated dramatically higher amounts of granzyme B exocytosis than did cells from CPs. This finding was confirmed by measuring the frequency of HIV-1-infected target cells that remained after coculture with stimulated effector cells. The enhanced cytotoxicity was seen even at very low effector to target cell ratios in HCs whereas it was minimal at even high effector to target ratios in CPs. Thus the enhanced cytotoxicity was not simply the result of a greater number of HIV-1-specific effectors in HCs as a result of enhanced proliferation that occurred during the

stimulation process. It was also not due to activation-induced cell death of effector cells from CPs, as indicated by the fact that these cells could still secrete large amounts of IFN-γ. The granzyme B-mediated killing occurred within one hour, which is important because productively infected $CD4^+$ T cells have a half-life of less than 1 day. It follows that the effector cells could kill infected targets before HIV-1 could complete its life cycle and release virions that could perpetuate the infection.

Prior studies have shown that HIV-1-specific $CD8^+$ T cells from CPs have a block in proliferation and thus could be considered partially anergic (Migueles et al., 2002). This block is not a direct consequence of HIV-1 viremia alone because it is not reversed by treatment with a potent cocktail of antiretroviral drugs known as highly active antiretroviral therapy (HAART). Interestingly, this study shows that the block in proliferation of HIV-1-specific $CD8^+$ T cells can be overcome by treatment with phorbol-12-myristate 13-acetate (PMA) and ionomycin. After a period of rest, restimulation with HIV-1 antigens results in effectors from CPs that are as good at granzyme B-mediated killing of target cells as effector cells from HCs.

The data from this study define at least one mechanism responsible for $CD8^+$ T cell-mediated suppression of HIV-1 replication, but there are many more questions that need to be addressed. Why do some patients become HCs and others CPs even when they share the same protective HLA alleles? Is this just a consequence of differences in their ability to develop effective killer cells? Is the impressive granzyme B-mediated killing seen in HCs limited to just HIV-1-specific $CD8^+$ T cells or is it a more general phenomenon? If HIV-1 viremia is not the cause of the partial anergy seen in CPs, what is? Studies have shown that HIV-specific $CD8^+$ T cells are clearly exerting selective pressure on HIV-1 in HCs (Bailey et al., 2006). Why then is this granzyme B-mediated killing not seen in freshly isolated cells from HCs when these cells are presumably responsible for the control of HIV-1-infected cells in vivo? Why does proliferation appear to be a requirement for generating effective killer cells that are capable of releasing granzyme B? It will take a while before all this is sorted out, but this study by Migueles et al. (2008) provides reason for optimism. They have described an important $CD8^+$ T cell function that correlates with immune protection. It will be important to test the ability of T cell-directed candidate vaccines to induce granzyme B-mediated killing of target cells. The finding that the stimulation of CPs cells with agonists that induce proliferation results in effective HIV-1-specific killers is an important proof of concept study that suggests that the partial anergy of HIV-1-specific $CD8^+$ T cells is reversible. Thus, therapeutic vaccination of CPs might eventually be a feasible goal.

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